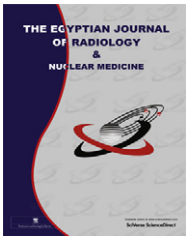
The Egyptian Journal of Radiology and Nuclear Medicine (2012) **43**, 257–264

Egyptian Society of Radiology and Nuclear Medicine

The Egyptian Journal of Radiology and Nuclear Medicine

www.elsevier.com/locate/ejrnm
www.sciencedirect.com



ORIGINAL ARTICLE

Clinical use of H^1 MR spectroscopy in assessment of relapsing remitting and secondary progressive multiple sclerosis

Hossam M. Abd El-Rahman [a], Doaa I. Hasan [a,*], Heba A. Selim [b], Sbah M. Lotfi [b], Wael M. Elsayed [b]

[a] *Department of Radio-Diagnosis, Zagazig University, Egypt*
[b] *Department of Neurology, Zagazig University, Egypt*

Received 30 September 2011; accepted 28 December 2011
Available online 9 February 2012

KEYWORDS

Multiple sclerosis;
RRMS;
SPMS;
EDSS;
Brain metabolites;
H^1 MR spectroscopy

Abstract *Background:* Conventional MRI has a limited ability to provide specific information about axonal pathology in MS, recently, MRI spectroscopy used for assessment of the axonal loss even in normal appearing white matter.
Objective: To assess the axonal degeneration in plaques and normal appearing white matter in patients with relapsing remitting MS and secondary progressive MS, and correlate their clinical disability using expanded disability status scale (EDSS) score with H^1 MRS abnormalities.
Patients and methods: Thirty-two MS patients (20 RRMS, 12 SPMS) and 20 controls were subjected to thorough history taking, clinical examination with special attention to: age at first symptoms, disease duration and the EDSS score. MRS was performed in order to map *N*-acetylaspartate (NAA), choline (Cho) and creatine (Cr).

* Corresponding author. Address: Zagazig University Hospital, Sharkia, Egypt. Tel.: +20 113694905.
E-mail addresses: hossammansour72@hotmail.com (H.M. Abd El-Rahman), dididodge@yahoo.com (D.I. Hasan), habhoob_444@hotmail.com (H.A. Selim), Ahmed.nader51@yahoo.com (S.M. Lotfi), Drwaelneuro@yahoo.com (W.M. Elsayed).

Peer review under responsibility of Egyptian Society of Radiology and Nuclear Medicine.
doi:10.1016/j.ejrnm.2011.12.009

Results: In SPMS, the NAA/Cr ratio and absolute concentrations for NAA in MS plaques and NAWM were significantly reduced compared to RRMS and to controls, also, significant relation with this metabolite values and clinical disability using EDSS score.
Conclusion: In SPMS patients group there were significant reduction in the levels of NAA in both plaques and NAWM compared to RRMS and control groups, moreover significant correlation of NAA reduction in the plaques of both groups related to clinical disability and disease progression.

1. Introduction

Multiple sclerosis (MS), one of the most common causes of neurological disability in young adults, is an inflammatory demyelinating and degenerative autoimmune disorder of the central nervous system (CNS) (1).

Several patterns of the disease have been delineated. The most common classification includes benign (20%), relapsing remitting MS (RRMS) (25%), secondary progressive MS (SPMS) (40%), primary progressive and other forms (15%) (2).

Although conventional MRI is sensitive in detecting the lesions of multiple sclerosis, it disclosed neither demyelination nor axon loss itself (3), also, the amount of white matter involvement seen on FLAIR and T2-weighted MR images is poorly related to clinical disability (4). Proton magnetic resonance spectroscopy (MRS) is a nuclear magnetic resonance technique which has the potential to detect axon loss, and provide tissue metabolic information in vivo (5). Hallmark of all subtypes of MS are focal lesions that are distinguished by their content of activated macrophages and T cells, edema, demyelination, and axonal transection (6), each of these histopathologies appears hyperintense on T2-weighted MRI. The white matter, which appears normal on gross pathology, shows mostly astroglial hypertrophy and to a much lesser extent inflammatory infiltrate, demyelination, or axonal loss (7).

MRS studies (5,8,9) comparing lesions and normal appearing white matter (NAWM) in RRMS and SPMS measured the following metabolites: *N*-acetyl aspartate (NAA), which has been suggested to be a marker for neuron viability and axonal density in the brain (10), creatine (Cr) which represents a combination of creatine and phosphocreatine, a marker of gliosis (11,12) and choline (Cho) thought to be a marker associated with membrane phospholipids, in which greater concentrations of choline are highly indicative of active inflammatory disease (13). H^1 MR studies of RRMS show reduced NAA/Cr in T2-intense lesions and normal appearing white matter (10).

Presumably, these metabolic changes in normal appearing white matter reflect microscopic disease and decreased NAA/Cr in lesions and normal appearing white matter of RRMS probably reflects decreased NAA and increased Cr (11,14). NAA is shown to be similarly reduced in MRI lesions of SPMS and RRMS and NAA is also decreased in normal appearing white matter in SPMS (8).

Previous studies have reported that Cr is increased in RRMS normal appearing white matter compared to white matter of SPMS. The increased Cr has been attributed to microscopic disease; most likely gliosis. The reduction in NAA is the reflection of more disability as in SPMS group. If increase in Cr in RRMS normal appearing white matter and focal lesions relative to SPMS would be consistent with the notion that sustained progression of disability reflects gliosis in addition to inflammation and axonal loss (8,15).

The aim of this study was to assess the role of H^1 MRS in the extent of axonal degeneration in NAWM in patients with RRMS and SPMS, and, to correlate their clinical data with H^1 MRS abnormalities.

2. Patients and methods

The present study was carried out on 32 multiple sclerosis patients from June 2009 to June 2011, who met the criteria of clinically definite MS according to Poser et al. (16) definitions, selected from Neurology Department, Zagazig University Hospitals, 20 patients with RRMS and 12 patients with SPMS. Twenty healthy volunteers with similar age and gender distribution (control group) were included in this study. The study was approved by the Ethical Committee of our Faculty and informed written consent was obtained from patients and controls.

All patients were subjected to:

I. Full general and neurological examination with special attention to:
   (1) The duration of the disease (i.e., the interval between the date of first symptom and the date of examination (measured in years)).
   (2) Patient's age at first symptoms ($\leqslant$25 years or $>$25 years).
   (3) The expanded disability status scale (EDSS) score(17):
   - Patients with RRMS were divided according to EDSS score to:
     - Mild disability $\leqslant$2.5.
     - Moderate to severe disability $>$2.5.
   - Patients with SPMS, few of them had mild disability and these patients were therefore reclassified with those with an EDSS score $\leqslant$5.5 and an EDSS score greater than 5.5 to provide a homogenous division.

II. Radiological evaluation was performed in Zagazig University Radiology MRI Unit: MR images were acquired using a 1.5T Philips (Achieva, class IIa) using a standard quadrature head coil. The following sequences were collected from each patient and control during a single session:
   * T1 weighted Sagittal localizer (TE = 10 ms, TR = 500 ms).

* Axial T1 (TE = 15 ms, TR = 450 ms, FOV = 22 cm, matrix = 256 × 128).
* Axial T2 (TR = 6000, TE = 100 ms, FOV = 22 cm, thickness = 5 mm, matrix = 256 × 128).
* Axial and Sagittal FLAIR(fluid-attenuation inversion recovery) (TR = 800, TE = 147 ms, FOV = 22 cm, thickness = 5 mm, matrix = 256 × 128).
* Three dimensional proton spectroscopic imaging was performed with a repetition time (TR) of 1500 ms and an echo time (TE) of 135 ms using single voxel MRS. The number of peaks fitted included the chemical shift ranges:
  - *N*-acetylasparate (NAA) at 2.0 ppm.
  - Creatine/phosphocreatine (Cr) at 3.0 ppm.
  - Choline compounds (Cho) at 3.2 ppm.
  - Myo-inositol (mI) at 3.5 ppm.
  - Glutamine–glutamate–GABA complex (Glx) between 2.1 and 2.5 ppm.
  - Lactate (Lac) 1.35 ppm.
  - Free lipids (Lip): wide resonance at 1.3 and 0.9 ppm.

Using a standardized post-processing protocol, the raw data were thus processed automatically, allowing for operator-independent quantifications. Metabolite concentrations were determined from peak areas. As many literature (10,13) the peak values of *N*-acetylaspartate (NAA), choline (Cho) and creatine (Cr) and relative ratios of these metabolites were calculated.

2.1. Image interpretations

These were analyzed for a variety of radiographic features:

- FLAIR-T2WIs for detection of the location and lesion actual size.
- The ROIs within MS plaques and within normal white matter.
- The ROIs within normal white matter of control group.
- The ROIs may be located anterior or posterior to centrum semiovale.

2.2. Statistical analysis

We performed all statistical analyses with the SPSS version 11, $P < 0.05$ was considered statistically significant.

Group means were compared using unpaired Student's *t*-test and one way analysis of variance (ANOVA) test. Pearson correlation coefficient (*r*) was calculated to assess the correlation between quantitative data (18).

The significance of the NAWM metabolite concentrations between both patient groups and the healthy controls was assessed by the Mann Whitney test.

3. Results

Demographic and clinical data of our patients were illustrated in Table 1: This study included two groups of patients with MS, divided according to the course of the disease into:

The first group: 20 patients with RRMS, seven males, 13 females. Their mean age was 38 ± 8.9 years, the mean disease duration was 5.8 ± 2.3 years, EDSS score had a mean of 1.65 ± 2.1 and the mean age at first symptom was 29.8 ± 10.1 years.

The second group: 12 patients with SPMS, five males, seven females, their mean age was 43.1 ± 11.1, the mean disease duration was 10.5 ± 5.8, EDSS score had a mean of 4.95 ± 1.85 and the mean age at first symptom was 33 ± 11.2 years.

The control group: 20 volunteers, 10 males and 10 females, with mean age 42.1 ± 10.5 years.

MRI findings in our patients were listed in Table 2, supra-tentorial lesions present in 28 patients, eight of them were periventricular and ranging from 0.4 to 2 cm, while 20 lesions were cortical and subcortical and ranging from 0.2 to 1 cm. Only four patients had infra-tentorial lesions ranging from 0.4 to 2 cm.

In SPMS patients we found that there was a significant reduction of NAA in MS plaques (Fig. 1) which were (3.97 ± 1.47).

When comparing neurometabolites, the level of NAA in NAWM of the SPMS group, there was a significant reduction of NAA (Fig. 2) comparing with RRMS and controls. NAA (6.5 ± 1.9 versus 7.48 ± 2.1 and 8.9 ± 1.85, respectively, $p \leqslant 0.05$) as shown in Table 3.

The other finding noted in this study was the significant reduction in both NAA/Cr and NAA/Cho ratios in SSPM plaques and NAWM compared to RRMS and controls (in SPMS were 1.68 ± 1.3 and 1.38 ± 0.65 versus 3.74 ± 1.4, 3.1 ± 1.1 and 4.1 ± 1.62, 3.2 ± 1.2 in RRMS and controls, respectively, $p \leqslant 0.05$).

Increased Cr and Cho in the RRMS (Fig. 3) but they were not statistically significant compared to the SPMS and controls.

When we correlated NAA/Cho ratio and age at first symptoms, we found that there was a significant reduction in the ratios in patients with RRMS whose age at first symptom was >25 years, but in patients with SPMS there was a significant reduction in ratios in patients whose age at first symptoms was <25 as shown in Table 4. Also, there is a positive significant relation between longer disease duration of both groups and the reduction of metabolite ratio.

Regarding the relation between MRS findings (NAA/Cho ratio) and clinical disability using EDSS score we found that there is negative significant correlation between patients with RRMS (EDSS score ⩽2.5) (B-coefficient = −0.46, SE = 0.06, $P \leqslant 0.05^*$), also, those with moderate to severe disability (in SPMS, EDSS score >5.5) (B-coefficient = −0.46, SE = 0.06, $P \leqslant 0.05^*$) (Table 5).

4. Discussion

Advanced MRI techniques including MR spectroscopy have been shown to provide information about structural and biochemical alterations within and outside MS WM lesions (19,20). Also, MRS has proven useful in assessing neuronal damage like axonal loss, as NAA is exclusively localized in the neuronal compartment (21,22). As, axonal and neuronal damages have long been implicated as a main cause of irreversible MS disability (23). NAA is almost exclusive to neurons and correlated better with clinical disability than other imaging metrics (24,25), as it is an early event that occurs before the formation of MRI visible lesions (5).

Table 1 Clinical data of the two groups as classified by clinical disease course, and control group.

	RRMS (n = 20)	SPMS (n = 12)	Control (n = 20)
Age (years)	39 ± 8.9	43 ± 11.1	42.1 ± 10.5
M/F	7/13	5/7	10/10
Disease duration	5.8 ± 2.3	10.5 ± 5.8	–
EDSS score	1.65 ± 2.1	4.95 ± 1.85*	–
Age at first symptom (years)	29.8 ± 10.1	32 ± 11.0	–

* Statistically significant relationships, $P \leqslant 0.05$.

Table 2 Imaging finding in our patients by conventional MRI technique.

Location (No.)	Size in FLAIR (No.)	MR appearance
Supra-tentorial (28)	04–2 cm (8)	Oval and linear lesions around the periventricular area, perpendicular to the long axis of the ventricles
	0.2–1 cm (20)	Rounded small lesions in the cortical and subcortical regions
Infra-tentorial (4)	2–3 cm (2)	Ill-defined lesions in parietal white matter
	0.4–2 cm (2)	Oval and rounded shape cerebellar peduncles and brain stem lesions

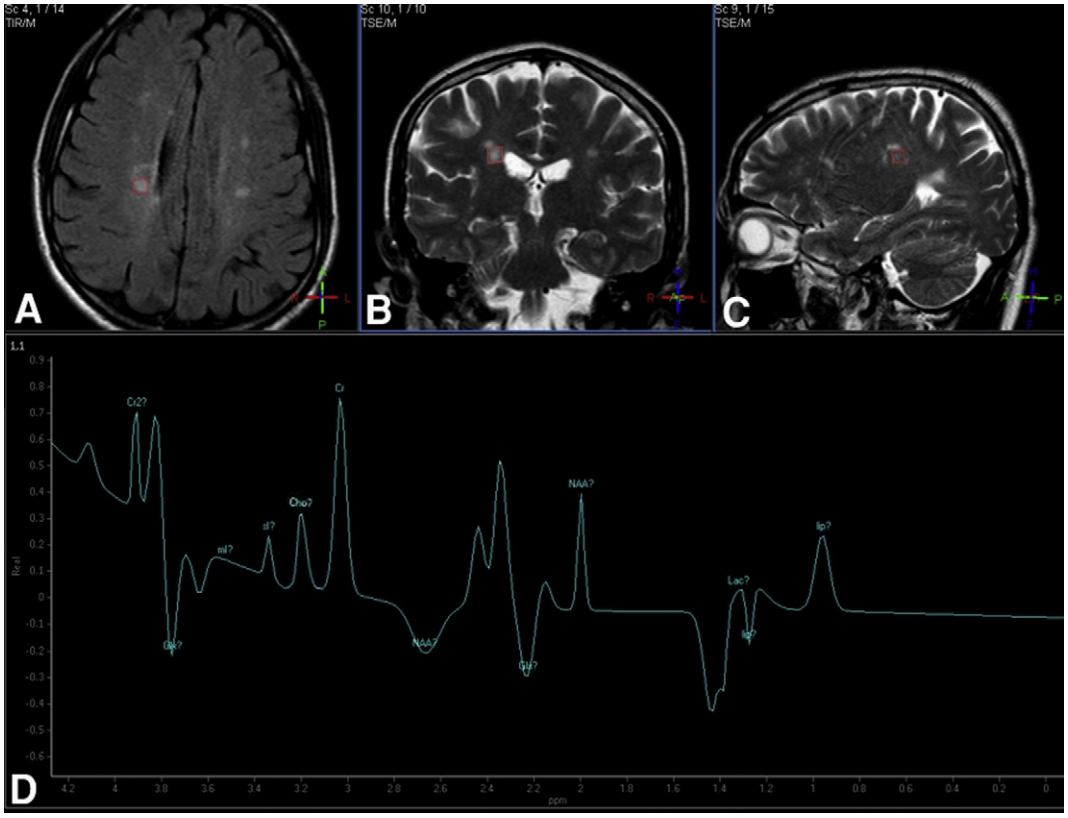


Figure 1 MR images of a 34-year-old male SPMS patient. (A) Axial FLAIR. (B) Coronal T2 W. (C) Sagittal T2W showing multiple periventricular and subcortical MS plaques in all images, the red rectangles indicate the volume of interest (VOI) for MR spectroscopy. (D) Proton MR spectra of this SPMS patient showing reduction of *N*-acetylaspartate (NAA), choline (Cho) and elevated creatine (Cr) peaks.

In our study, we studied the clinical disability of our two patient groups, using EDSS score; also we analyzed NAA, Cr and Cho levels of MS plaques and NAWM of 32 MS patients and 20 controls.

In the present study, negative significant correlation between MRS finding and age at first symptom, disease duration, this finding is in agreement with previous studies (26–29). In the current study, we found a positive significant correlation between MRS findings and clinical score using EDSS score (-0.46, $P \leqslant 0.05^*$), in accordance with findings reported by Adalsteinsson et al. (30). MRS seems to be a potential surrogate marker of disease progression (26). In contrast to our

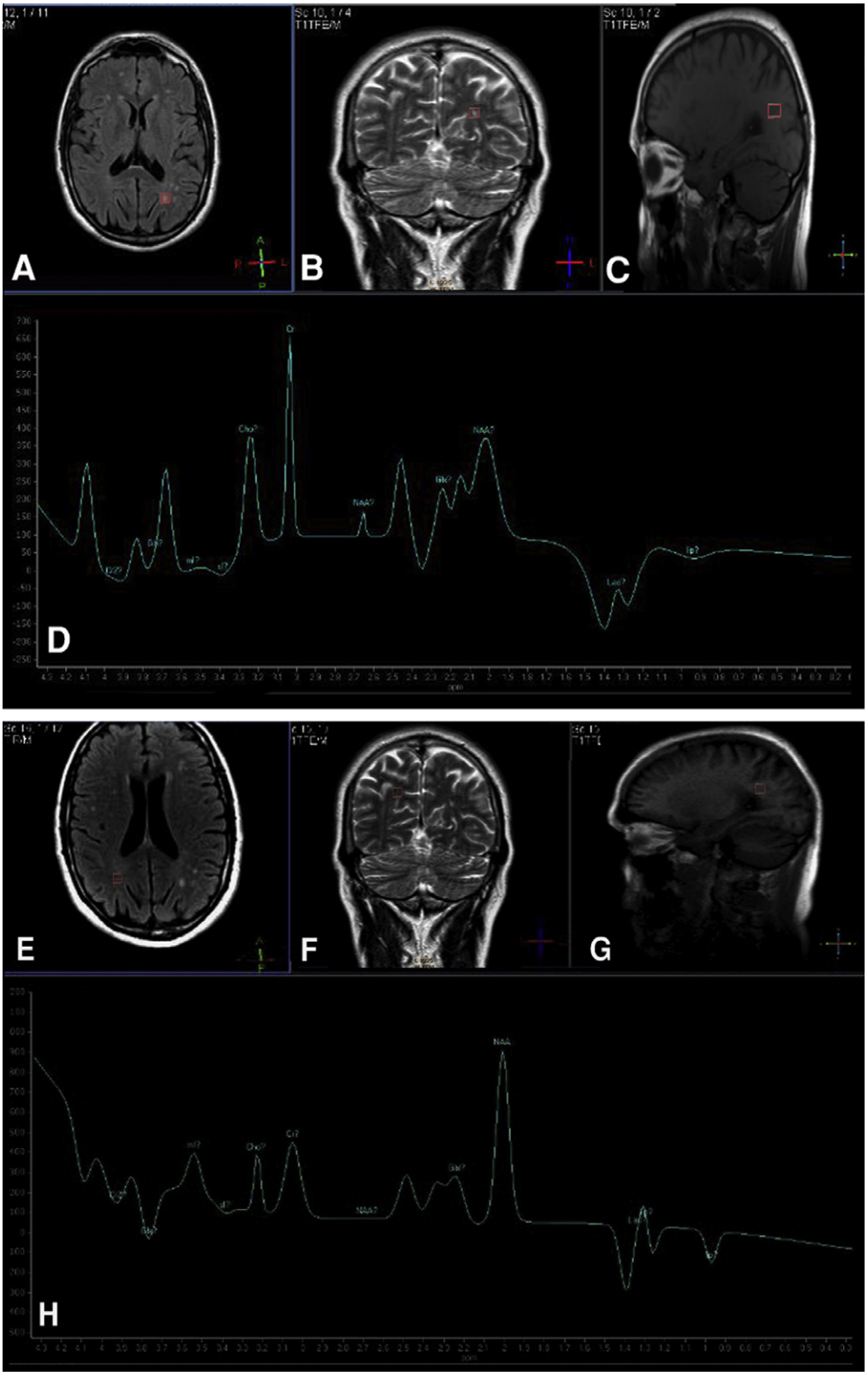


Figure 2 MR images of a 28-year-old male patient with SPMS. (A) Axial FLAIR. (B) Coronal T2WI. (C) Sagittal T1WI showing multiple periventricular MS plaques, the red rectangles indicate the volume of interest (VOI) for MR spectroscopy. (E) Axial FLAIR. (F) Coronal T2WI. (G) Sagittal T1WI of the same patient where the red rectangles in the normal white matter area in the Rt. side. (D and H) proton MR spectra of this SPMS patient showing reduction of *N*-acetylaspartate (NAA), choline (Cho) and elevated creatine (Cr) peaks in plaque as compared with the normal appearing white matter of the other cerebral hemisphere.

Table 3 Comparison between the MRS different metabolites values in MS plaques of both RRMS and SPMS, contralateral normal appearing white matter and healthy controls white matter.

	NAA, X ± SD	Cr, X ± SD	Cho, X ± SD	NAA/Cr, X ± SD	NAA/Cho, X ± SD
RRMS (n = 20)					
• MSP	5.48 ± 2.3	4.06 ± 1.8	2.5 ± 0.5	3.74 ± 1.4	3.1 ± 1.1
• NAWM	7.48 ± 2.1	5.1 ± 2.1	2.48 ± 0.4	3.84 ± 1.53	3.3 ± 1.2
SPMS (n = 12)					
• MSP	3.97 ± 1.47*	2.45 ± 1.0*	2.52 ± 0.56*	1.68 ± 1.3*	1.38 ± 0.65*
• NAWM	6.5 ± 1.9*	3.4 ± 1.5	2.47 ± 0.49	2.6 ± 1.1	2.8 ± 0.7
Control (n = 20)	8.9 ± 1.85	4.56 ± 1.43	2.49 ± 0.6	4.1 ± 1.62	3.2 ± 1.2

X = mean (data are mean concentrations mmol/L) ± standard deviations; SD = standard deviation.
* Statistically significant relationships, $P < 0.05$.

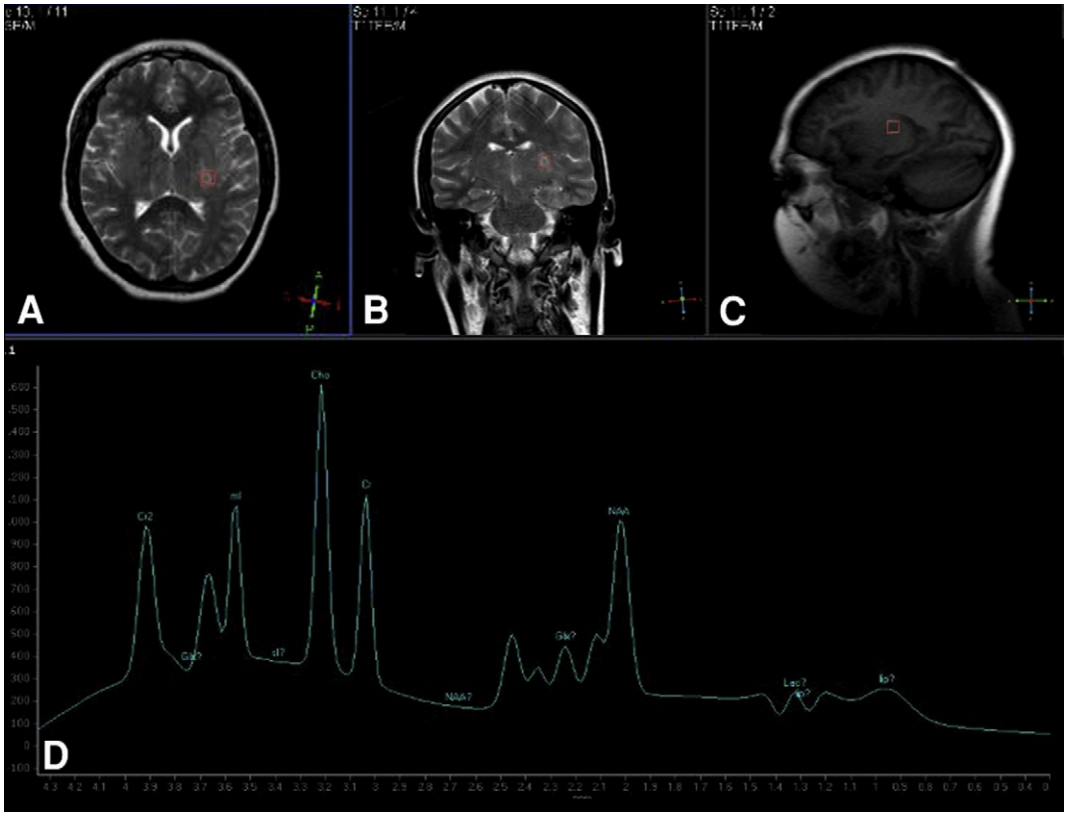


Figure 3 MR images of a 17-year-old female RRMS patient. (A) Axial T2WI. (B) Coronal T2WI. (C) Sagittal T1WI showing left parietal subcortical MS plaque in all images, the red rectangles indicate the volume of interest (VOI) for MR spectroscopy. (D) Proton MR spectra of this RRMS patient showing relative preservation of (NAA), elevated Cr, which representing glial cell metabolism and tissue repair mechanisms and high choline (Cho) representing active inflammation (de- and re-myelination).

Table 4 The relation between NAA/Cho ratio in brain and age at first symptoms, disease duration in both groups.

	RRMS		SPMS	
	r	P-value	r	P-value
Age at first symptoms	+0.49	<0.05*	0.485	<0.05*
Disease duration	+0.51	<0.05*	0.55	<0.05*

r = correlation coefficient.
* Statistically significant relationships, $P < 0.05$.

findings, recently, Rigotti et al. (9), who studied whole brain NAA (WBNAA) in 43 patients with RRMS, found that no correlation between WBNAA and age of onset, disease duration nor clinical disability using EDSS score, they also found that no significant difference in WBNAA when comparing genders or patients receiving treatment versus those who have not.

Proton MR spectroscopy is a well-established method for the in vivo investigation of the normal appearing white matter in patients with MS (29).

Table 5 The relation between NAA/Cho ratio in brain and clinical disability using EDSS score.

Independent variable	Mean correlation efficient	SE	*P*-value
EDSS (RRMS)	−0.46	0.06	$<0.05^*$
EDSS (SPMS)	−0.44	0.045	$<0.05^*$

SE = standard error.
* Statistically significant relationships, $P < 0.05$.

In our study, MR spectroscopy revealed significant changes in SPMS, NAA/Cr of MS plaques and NAWM in comparison with RRMS and healthy controls, most probably reflecting different pathophysiological mechanisms such as axonal damage (31).

Recent surveys underline the potential of MRS in elucidating widespread tissue damage in NAWM, monitoring various aspects of inflammation in MS, characterizing new, stable and resolved lesions, predicting the individual clinical course and monitoring treatment – related effects on myelin repair and neuroprotection (20,32–34).

Our finding observed significant decrease in NAWM NAA concentration (and a correspondingly reduced NAA/Cr ratio) in patients with RRMS and SPMS, our results matched with previous studies (35–37).

In contrast to our results, Fernando et al. (38) reported higher absolute concentrations of NAA in NAWM and no significant decreased NAA level in patients with definite MS.

A reduction of NAA levels in MS patients was correlated with secondary axonal loss in chronic lesions, representing irreversible damage to the central nervous system (39).

Although the Cr and Cho were increased in RRMS group in plaques and NAWM, there was not statistically significant when compared to SPMS and the controls. These are consistent with the notion that sustained progression of gliosis and inflammatory changes (demyelination–remyelination). On contrary, other authors (40) found in their study significant elevation in Cr in RRMS group compared to controls.

Increased Cho levels in MSP and NAWM in RRMS, as well as decreased NAA concentrations, is in accordance with other studies (26,41), presenting inflammatory changes and significant axonal damage.

Metabolic ratios are less sensitive than absolute concentrations of individual metabolites (42,43). When comparing NAA/Cho and NAA/Cr ratios in the lesions, NAWM in patients versus controls, they were clearly reduced, consistent with results obtained in previous studies (11,44,45). The observation is that the magnitude of the reduction varies with the type and clinical form of the disease. Our comparison of NAA/Cho and NAA/Cr ratios in patients showed that the reduction in NAA was more evident in SPMS patients in comparison with RRMS and controls.

5. Conclusion

Our findings support that axonal loss represented by metabolites concentrations changes in MRS for both SPMS and RRMS is important in the development of the disease disability in multiple sclerosis. They also provide evidence for axonal loss in normal appearing white matter in patients with SPMS. Further studies with larger cohorts of patients are necessary to clarify the significance of this method in monitoring therapeutic efficacy.

References

(1) Compston A, Coles A. Multiple sclerosis. Lancet 2002;359:1221–31.
(2) Lublin FD, Reigold S. For the National Multiple Sclerosis (USA) Advisory Committee Trials of New Agents in Multiple Sclerosis. Defining the clinical course of multiple sclerosis: results on an international survey. Neurology 1996;64:907–11.
(3) Ge Y. Multiple sclerosis: the role of MR imaging. AJNR 2006;27:1165–76.
(4) Koopmans RA, Li DKB, Grochowsky E, Culter PJ, Paty DW. Benign versus chronic progressive multiple sclerosis: magnetic resonance features. Ann Neurol 1989;25:74–81.
(5) Sajja BR, Wolinsky JS, Narayana PA. Proton magnetic resonance spectroscopy in multiple sclerosis. Neuroimaging Clin N Am 2009;19:45–58.
(6) Raine C, Dale E, McFarlin A. The memorial lecture: the immunology of the multiple sclerosis lesions. Ann Neurol 1994;36:S61–72.
(7) Allen IV, McKeown SR. A histological, histo-chemical and biochemical study of the macroscopically normal white matter in multiple sclerosis. J Neurol Sci 1979;41:81–91.
(8) Davie CA, Barker GJ, Thompson AJ. Magnetic resonance spectroscopy of chronic cerebral white matter lesions and normal appearing white matter in multiple sclerosis. J Neurol Neurosurg Psychiatry 1997;63:736–42.
(9) Rigotti DJ, Gonen O, Grossman R, Babb JS, Falini A, Benedetti B, et al. Global *N*-acetyl-aspartate declines in benign multiple sclerosis. AJNR 2011;32:204–9.
(10) Urenjak J, Williams SR, Gadian DG, Noble M. Specific expression of *N*-acetyl aspartate in neurons, oligodendendrocyte-type-2 astrocyte progenitors, and immature oligodendrocytes in vitro. J Neurochem 1992;59:55–61.
(11) Arnold DL, Matthews PM, Francis G, Antel J. Proton magnetic spectroscopy of human brain in vivo in the evaluation of multiple sclerosis: assessment of the load of disease. Magn Reson Med 1990;14:154–9.
(12) Urenjak J, Williams SR, Gadian DG, Noble M. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. J Neurosci 1993;13:981–9.
(13) Jagannathan NR, Desai NG, Raghunathan P. Brain metabolite changes in alcoholism: an in vivo proton magnetic resonance spectroscopy (MRS) study. Magn Reson Imaging 1996;14:553–7.
(14) Husted CA et al. Biochemical alterations in multiple sclerosis lesions and normal appearing white matter detected by in vivo 31P and 1H spectroscopic imaging. Ann Neurol 1994;36:157–65.
(15) Suhy J, Rooney WD, Goodkin DE, Capizzano AA, Soher BJ, Maudsley AA, et al. H ^{1}MRSI comparison of white matter and lesions in primary progressive and relapsing remitting MS. Mult Scler 2000;6:148–55.
(16) Poser CM, Paty DW, Scheinberg L. New diagnostic criteria of multiple sclerosis: guidelines for research protocols. Ann Neurol Clin 1983;103:227–31.
(17) Kurtzke JF. Rating neurologic impairment in multiple sclerosis an expanded disability status scale (EDSS). Neurology 1983;13:227–31.
(18) Dean A, Dean J, Coulonpier D, et al. Epi-info (version 6.02) a world processing data-base and statistical progression for epidemiology on micro computer CDC, Atanta, Gorgia, USA, 1994.
(19) Bakshi R, Thompson AJ, Rocca MA. MRI in multiple sclerosis. Current status and future prospects. Lancet 2008;7:615–25.
(20) Zivadinov R, Stosc M, Cox J, et al. The place of conventional MRI and newly emerging MRI techniques in monitoring different aspects of treatment outcome. J Neurol 2008;255:61–74.
(21) Li DK, Held U, Petkau J, Daumer M, Barkhof F. MRI T2 lesion burden in multiple sclerosis: a plateau relationship with clinical disability. Neurology 2006;66:1384–9.

(22) Simmons ML, Frondoza CG, Coyle JT. Immunocytochemical localization of *N*-acetyl-aspartate with monoclonal antibodies. Neuroscience 1991;45:37–45.
(23) Arnold DL, De Stefano N, Narayanan S, Matthews PM. Axonal injury and disability in multiple sclerosis: magnetic resonance spectroscopy as a measure of dynamic pathological change in white matter. In: Filippi M, Arnold DL, Comi GC, editors. Magnetic resonance spectroscopy in multiple sclerosis. Milan, Italy: Springer; 2001. p. 61–7 [2004;46:15–21].
(24) Benarroch EE. *N* acetyl aspartate and *N*-acetylaspartyglutamate: neurobiology and clinical significance. Neurology 2008;70:1353–7.
(25) Mathiesen HK, Jonsson A, Lannucci G. Correlation of global *N* acetyl aspartate with cognitive impairment in multiple sclerosis. Arch Neurol 2006;63:533–6.
(26) Bellman-Strbl J, Stiepani H, Wuerfel J, Paul F, Warmut C, Aktas O, et al. MR spectroscopy and magnetization transfer imaging, lesion load and clinical scores in early relapsing remitting multiple sclerosis: a combined cross sectional and longitudinal study. Eur Radiol 2009;19:2066–74.
(27) Khaleeli Z, Sastre-Garriga J, Ciccarelli O. Magnetisation transfer ratio in the normal appearing white matter predicts progression of disability over 1 year in early primary progressive multiple sclerosis. J Neurol Neurosurg Psychiatry 2007;10:1076–82.
(28) Wattjes MP, Harzheim M, Lutterbey GG. Prognostic value of high-field proton magnetic resonance spectroscopy in patients presenting with clinically isolated syndromes suggestive of multiple sclerosis. Neuroradiology 2008;50:123–9.
(29) Wattjes MP, Harzheim M, Lutterbey GG, Klotz L, Schild HH, Traber F. Axonal damage but no increased glial cell activity in the normal appearing white matter of patients with clinically isolated syndromes suggestive of multiple sclerosis using high-field magnetic resonance spectroscopy 2007;28:1517–22.
(30) Adalsteinsson E, Langer-Gould A, Homer J. Gray matter *N*-acetyl aspartate deficits in secondary progressive but not relapsing remitting multiple sclerosis. AJNR 2003;10:1941–5.
(31) De Stefno N, Guidi L, Strmillo ML. Imaging neuronal and axonal degeneration in multiple sclerosis. Neurology 2003;24: S283–6.
(32) Zivadinov R. Can imaging techniques measure neuroprotection and remyleination in multiple sclerosis? Neurology 2007;68: S72–82.
(33) Giacomini PS, Arnold DL. Non-conventional MRI techniques for measuring neuroprotection, repair and plasticity in multiple sclerosis. Curr Opin Neurol 2008;21:272–7.
(34) Rovira A, Leon A. MR in the diagnosis and monitoring of multiple sclerosis: an overview. Eur J Radiol 2008;67:409–14.
(35) Sastre-Garriga J, Ingle Gt, Chard DT. Metabolic changes in normal appearing gray and white matter are linked with disability in early primary multiple sclerosis. Arch Neurol 2005;62: 569–73.
(36) Vrenken H, Barkhof F, Uitdehaag BM. Mr spectroscopic evidence of glial but not for neuro-axonal in MS normal appearing white matter. Magn Reson Med 2005;53:256–66.
(37) Srinivasan R, Sailasuta N, Hurd R, It AL. Evidence of elevated glutamate in multiple sclerosis using magnetic resonance spectroscopy at 3 T. Brain 2005;128:1016–625.
(38) Fernando KTM, McLean MA, Chard DT, et al. Elevated white matter myoinositol in clinical isolated syndromes suggestive of multiple sclerosis. Brain 2004;127:1361–9.
(39) Falini G, Calabrese Mm Fillipi D, Origgi S, Lipari B, Colombo G, Comi G, et al. Benign versus secondary progressive multiple sclerosis: the potential role of proton MR spectroscopy in defining the nature of disability. AJNR 1998;19:223–9.
(40) Pan JW, Coyle PK, Bashir K, Hetherington PH. Spectroscopy metabolic differences between multiple sclerosis subtypes measured by quantitative MR. Mult Scler 2002;8:200–8.
(41) Inglese M, Li BS. Diffusely elevated cerebral choline and creatine in relapsing remitting multiple sclerosis. Magn Reson Med 2003;50:190–5.
(42) Wattjes MP, Lutterbey GG, Harzheim M, Gieseke J, Traber F. Higher sensitivity in the detection of inflammatory brain lesions in patients with clinically isolated syndromes suggesting of multiple sclerosis using high field MR: an intraindividual comparison between 1.5 T with 3 T. Eur Radiol 2006;16:2067–73.
(43) Brief EE, Moll R, Li DK, Mackay AL. Absolute metabolite concentrations calibrated using the total water signal in brain H^1MR spectroscopy. NMR 2009;22:349–54.
(44) Arnold DL, Matthews PM, Francis GS, O'Connor J, Antel J. Proton magnetic resonance spectroscopic imaging for metabolic characterization of demyelinating plaques. Ann Neurol 1992;31:235–41.
(45) Kirov II, Patil V, Babb JS, Rusinek H, Herbert J. MR spectroscopy indicates diffuse multiple sclerosis activity during remission. J Neurol Neurosurg Psychiatry 2009;80:1330–6.